

Effect of combine application of Urea, Urease, Nitrification Inhibitors and Plant Growth Regulators on Spinach productivity

ABSTRACT: An experiment was conducted to assess the effect of applying different rates of urea treated with nBTPT (N-(n-butyl) thiophosphoric triamide) alone, or a combination of both nBTPT and DCD (Dicyandiamide) and DMPP (3, 4-Dimethylpyrazol-phosphate), on spinach productivity at experimental area of Faculty of Agriculture, Gomal University, Dera Ismail Khan during 2012-13. The experiment was arranged in a Complete Randomized Design (CRD) with nine treatments and four replications. Composite soil samples were collected before the experiment for various physico-chemical analysis viz soil texture, soil pH, organic matter, bulk density, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, extractable P and exchangeable K. The results showed that treatment (T6) with dose of Urea@150 kg/ha + GA3 @60 gram/ha was more effective than agrotain alone i.e tallest plant height (11.54cm), maximum number of leaves (69), highest fresh weight (22.37g), highest dry weight (4.64g), maximum biological yield (227g pot^{-1}) and highest net return (13.42 million). Such findings may be due to availability of nitrogen for longer period and reduced loss on nitrogen by volatilization. The results further suggested that applying urea treated with Gibberellic acid (GA3) or Agrotain significantly increased the spinach growth. However, combine application of Urea, Urease, Nitrification Inhibitors and Plant Growth Regulators on Spinach productivity was better than urease inhibitor alone (agrotain).

Key words: Agrotain, Gibberellic acid, nBTPT, Spinach,

INTRODUCTION

Spinach (*Spinacia oleracea*) is an annual member of Chenopodiaceae family and a valuable vegetable which contains nutrients such as iron, calcium, phosphorus, sulfur, potassium etc (Peivast, 2002). Urea is the predominant form of N fertilizer worldwide and also in Pakistan (ca. 70%), principally because of its lower cost per unit of N, and the convenience for transport, storage and spreading, attributes which result from its higher N content (46% N), in comparison to the 35, 26, 21, and 18% N of ammonium nitrate (AN), calcium ammonium nitrate (CAN), ammonium sulphate (AS) and di-ammonium phosphate (DAP), respectively. However, urea has been reported to have lower N response efficiency (NRE), where NRE is

31 defined as kg of additional dry matter (biomass and grain) produced per kg of applied N,
32 when compared to other ammonium- and nitrate-based fertilizers. This is especially so if urea
33 is applied under non-optimum soil moisture (very dry) and high temperature (above 20°C)
34 conditions. The lower NRE of urea has always been attributed to the wide range of N losses
35 (5% to 53% of the applied N) as ammonia (NH₃), when compared to AN, CAN, AS and DAP
36 (Saggar *et al.*, 2012). Of course, some N losses, like gaseous emissions of NH₃, nitric oxide
37 (NO), nitrous oxide (N₂O) and molecular N (N₂) and immobilization by microorganisms to
38 organic N, will be unavoidable because they are part of the natural N cycle and would occur
39 after application of any ammonium-based fertilizer or dairy farm effluents. Hence,
40 controlling the rate of all of these N losses is critical for improving the response efficiency of
41 all chemical fertilizers.

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43 During the past decade, researchers have developed a variety of management
44 practices and technologies to improve the NRE of urea. One such approach is to treat
45 granular urea with the urease inhibitor N-(n-butyl) thiophosphoric triamide (nBTPT - trade-
46 name Agrotain®). The nBTPT is typically applied at a range of 250 to 500 mg per kg of urea
47 in order to delay urea's hydrolysis by 7 to 10 days (Zaman *et al.*, 2008). Several field and
48 glasshouse studies have shown that treating urea with nBTPT (250 to 1,000 mg nBTPT per
49 kg of urea-N) has the greatest potential to improve NRE in many cropping systems (Soares *et*
50 *al.*, 2012). Similarly, urea can be treated with both nBTPT and nitrification inhibitors like
51 dicyandiamide (DCD) or 3, 4-dimethylpyrazol-phosphate (DMPP) have gained commercial
52 adoption in agricultural system. The soils of Pakistan are deficient in nitrogen (Shah *et al.*,
53 2012).

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55 Similarly, fertilizer use efficiency can be further improved if urea is applied with different
56 additives like plant growth hormones or regulators (Sturm *et al.*, 1994). The word ‘hormone’
57 came from Greek language, where it means ‘to stimulate’ or ‘to set in motion’. Plant
58 hormones/growth regulators (PGR) are a group of naturally occurring organic substances,
59 which have the ability to influence physiological processes such as plant growth,
60 differentiation and development, stress responses, cell division and reproductive activities
61 when applied at a very low concentrations. Among the major classes of PGRs, auxins
62 (generally represented by indole-3-acetic acid (IAA)), the gibberellins (GAs), and the
63 cytokinins (CKs) have the most potential to be used as plant growth regulators. Curtailing
64 processes with urease or nitrification inhibitors prolong the existence of nitrogen in soil
65 giving greater opportunity to plants for its utilization and hence reducing chances of its
66 volatilization (Ali *et al.*, 2012). The farmers are using nitrogenous fertilizers, mainly urea, to
67 remove its deficiency as crops respond strongly to these fertilizers (Ehsanullah *et al.*, 2012;
68 Ali and Noorka, 2013).

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70 However, limited information is available on the effect of urea treated with nBTPT
71 alone versus a combination of nBTPT and DCD or DMPP, and PGRs on plant productivity in
72 hot climate conditions in Pakistan. Therefore, the objectives of this study are to investigate
73 the effect of applying different rates of urea treated with nBTPT alone, or a combination of
74 both nBTPT and DCD and DMPP, on productivity of spinach. Owing to the importance of
75 spinach, and the effect of soil mineral nutrient content on its growth, yield and quality, this
76 experiment was conducted to evaluate the effect of manure and urea on yield, nutrient uptake
77 and nitrate accumulation in spinach.

MATERIALS AND METHODS

Pot experimental study was conducted at research farm of the Department of Soil and Environmental Sciences, Faculty of Agriculture, Gomal University, Dera Ismail Khan to assess the effect of applying different rates of urea treated with nBTPT alone, or a combination of both nBTPT and DCD and DMPP on spinach productivity. The experiment was laid out in Complete Randomized Design with following nine treatments containing four replications;

Treatments: Description

- | | |
|-----|---|
| T1: | No Nitrogen or GA ₃ (Control) |
| T2: | Urea only @150 kg N/ha |
| T3: | Agrotain treated Urea @150 kg/ha |
| T4: | Agrotain treated Urea @150 kg/ha + DCD@10 kg/ha |
| T5: | Agrotain treated urea @150 kg/ha + DMPP@2 kg/ha |
| T6: | Urea@150 kg/ha + GA ₃ @60 gram/ha |
| T7: | Agrotain treated Urea @150 kg/ha + GA ₃ @60 gram/ha |
| T8: | Agrotain treated urea@150 kg/ha + DCD @10 kg/ha + GA ₃ @60 gram/ha |
| T9: | Agrotain treated urea @150 kg/ha + DMPP@2 kg/ha + GA ₃ @60 gram/ha |

Experimental soil was crushed, passed through a 2 mm sieve and analyzed for different physical and chemical characteristics according to standard procedures. Such composite soil samples were collected before the experiment for various physico-chemical analysis viz soil texture, soil pH, organic matter, bulk density, NH₄-N, NO₃-N, extractable P and exchangeable K as shown in Table 1. In the diagnostic and research laboratory, the chemicals were used such as DCD (Powder) 10 kg per hectare, DMPP (Powder) 2 kg ha⁻¹, Agrotain Treated Urea 150 kg Nitrogen ha⁻¹, GA₃ 60 gram ha⁻¹ were used. In terms of agronomic

analysis, plant height (cm), number of leaves per plant, fresh and dry weight (g) and biological yield (g pot⁻¹) was also measured. In case of economic analysis, economic yield index as net return (Rs. in million) was calculated for its extensive commercial cultivation. Data recorded for tested parameters were statistically analyzed for Analysis of Variance (ANOVA) to find out the significance of the studied parameters. Means of the studied parameters were compared by least significant difference (LSD_{0.05}) using Advanced Statistix 10 computer program (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Plant Height (cm)

Results obtained on plant height of spinach as influenced by applying different rates of urea treated with nBTPT alone, or a combination of both nBTPT and DCD and DMPP, on spinach productivity are presented in Table 2. The findings showed that plant height varied significantly ($P < 0.05$) among the treatments. The shortest plant height (5.88 cm) in spinach plants recorded from control treatment (T₁) where no fertilizer was applied, whereas tallest plant height (11.54 cm) was noticed from treatment (T₆) amended with Urea@150 kg/ha + GA₃ @60 gram/ha, followed by treatments (T₅ and T₈) respectively that differed significantly from all other treatments. The value of Least Significant Difference (LSD) as 2.5, indicated significant impact of Urea@150 kg/ha + GA₃ @60 gram/ha on the plant height of spinach in agro-climatic conditions of Dera Ismail Khan. This may be due to availability of N for longer period and reduced loss on N by volatilization. Previous study conducted by Islam *et al.* (2011) pointed out that three types of organic manure alone produced moderate plant height and they were statistically similar with each other. Similar results findings were worked out by Zaman *et al.* (2009). Previous studies conducted by Ehsanullah *et al.* (2012); Ali and Noorka (2013) that farmers are using nitrogenous fertilizers, mainly urea, to remove its deficiency as crops respond strongly to these fertilizers.

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129 ***Number of Leaves per Spinach***

130 The results obtained on number of leaves per spinach as affected by applying different rates
131 of urea treated with nBTPT alone, or a combination of both nBTPT and DCD and DMPP, on
132 spinach productivity are presented in Table 2. The results revealed that number of leaves
133 were varied significantly ($P < 0.05$) among the treatments. The Minimum number of leaves
134 (26) in spinach plants were recorded from control treatment (T_1) where no fertilizer was
135 applied, while, maximum number of leaves (69) were recorded in spinach plants treated with
136 Urea@150 kg/ha + GA_3 @60 gram/ha followed by treatments (T_7 and T_2) respectively that
137 differed significantly from all other treatments. The value of Least Significant Difference
138 (LSD) as 15, showed highly significant impact of Urea@150 kg/ha + GA_3 @60 gram/ha on
139 the number of leaves of spinach in agro-climatic conditions of Dera Ismail Khan. The similar
140 findings of Akhtar *et al.* (2008) revealed that interaction between concentration of GA_3 and
141 different soaking times plants treated with 10ppm for 24 hours gave maximum number of
142 leaves per plant. Results findings of Babakhanzade *et al.* (2012) revealed that maximum
143 manure and urea application rates considerably enhanced yield of spinach; additionally the
144 interaction of 300 kg urea/ha $\times$ 45 ton manure/ha resulted in enhanced yield by about 54% as
145 compared to control.

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147 ***Fresh Weight (g) of Spinach***

148 The results obtained on fresh weight of spinach has showed that on average varied
149 significantly ($P < 0.05$) among the treatments in Table 2. The lowest fresh weight (8.52g) in
150 spinach plants recorded from control treatment (T_1) where no fertilizer was applied, while,
151 highest fresh weight of 22.37 g was attained in spinach plants amended with Urea@150 kg/ha

+ GA₃ @60 gram/ha followed by treatments (T₇ and T₅) respectively that differed significantly from all other treatments. The value of Least Significant Difference (LSD) as 4.16, indicating highly significant impact of different combinations especially rates of Urea@150 kg/ha + GA₃ @60 gram/ha on the dry weight of spinach in agro-climatic conditions of Dera Ismail Khan. Past study conducted by Wang *et al.* (2009) arrived at conclusion that any practice of maintaining a certain amount of NH₄⁺-N in soil condition is greatly useful to producing an enhanced fresh weight of spinach and optimized biological yield with high quality of spinach. Similar studies by Min-na *et al.* (2012) concluded that the shoot fresh mass may be employed important index for the evaluation of NUE changes in spinach genotypes at the same N level due to high co-efficiency of variation recorded at low and high N levels in spinach production.

Dry Weight (g) of Spinach

The results obtained on dry weight of spinach revealed significant variation (P<0.05) existed among treatments as affected by applying different rates of urea treated with nBTPT alone, or a combination of both nBTPT and DCD and DMPP. The lowest dry weight (3.40g) in spinach plants recorded from control treatment (T₁) where no fertilizer was applied, whereas, significant highest dry weight of 4.64 g was achieved on the application of Urea@150 kg/ha + GA₃ @60 gram/ha followed by treatments (T₉ and T₅) respectively that differed significantly from each other. The value of Least Significant Difference (LSD) as 0.7, indicated low level significant impact of Urea@150 kg/ha + GA₃ @60 gram/ha on the dry weight of spinach in agro-climatic conditions of Dera Ismail Khan. The findings of Wang *et al.* (2009) revealed a combination of N nutrition at a ratio of 25:75 in hydroponics cultivation of spinach would optimize the fresh and dry biomass yield and quality of spinach.

Bottom line in the experimental trial of spinach conducted in study is that Urea@150 kg/ha + GA₃ @60 gram/ha considerably improved the dry weight in spinach plants over moderate rate of N or urea alone. GA₃ is proved instrumental in promoting seed germination, elongation of stem, growth of flowering and fruit in various commercial plants. These materials have crucial role in improving nitrogen management of spinach plants. Similarly findings were worked out by Margreet *et al.* (2008) arrived at conclusion that net nitrate uptake rate (NNUR) efficiency considerably enhanced with increasing trend of RGR. Zaman *et al.*, (2008) also suggested reduced N losses via volatilization and enhanced pasture growth with combined application of urea and GA₃ as compared to urea alone.

Biological Yield (g pot⁻¹) of Spinach

The results obtained on Biological yield of spinach varied significantly ($P < 0.05$) among the treatments. The lowest biological yield (202 g plot⁻¹) in spinach plants recorded from control treatment (T₁) where no fertilizer was applied, whereas, highest biological yield of 277 g plot⁻¹ at treatment (T₆) was recorded in spinach plants amended with application of Urea@150 kg/ha + GA₃ @60 gram/ha followed by treatments (T₅ and T₃) respectively that differed significantly from all other treatments. The value of Least Significant Difference (LSD) worked out as 10.6, indicating overall significant impact of tested treatments especially highlighted dose of Urea@150 kg/ha + GA₃ @60 gram/ha on the biological yield of spinach grown in hot climatic environment of Dera Ismail Khan. Previous studies of Wang *et al.* (2009) were in association with present study which revealed a combination of N nutrition at a ratio of 25:75 in hydroponic cultivation of spinach would optimize the biomass yield and quality of spinach. Any practice of maintaining a certain amount of NH₄-N in soil condition is greatly effective in producing optimized yield with high quality of spinach. Moreover, the past research conducted by Babakhanzade *et al.* (2012) pointed out highest application of manure and urea application rates resulted in increased spinach yield; furthermore, the

interaction results (300 kg urea/ha × 45 ton manure/ha) also revealed enhanced yield by about 54% in comparison with control. The use of nitrogen inhibitors seems one of the promising strategies to reduce N losses from urea on calcareous alkaline soils provided other conditions are favorable such as soil organic matter and temperature (Ali *et al.*, 2012). Zaman *et al.* (2009) also showed that agrotain and dicyandiamide (DCD) in combination were more effective in minimizing NH₃ losses and N₂O emission, and in controlling urea hydrolysis, improving pasture production and retaining N in NH₄⁺ form. Other researchers also observed similar results whereas the combined use of nitrification and urease inhibitors reported better results in terms of increasing crop yields than their use alone (Dawar *et al.*, 2010; Nasima *et al.*, 2010).

Economic Analysis of Spinach Yield

The computation of economic analysis of spinach production in Table 3 revealed that on average maximum Net Return (13.42 Million) was achieved by Treatment (T₆), followed by Treatments (T₅ and T₃) respectively. Hence that treatment (T₆) with dose of Urea@150 kg/ha + GA₃ @60 gram/ha in view of highest net return proved economical and financially feasible treatments for profitable production of spinach in hot climatic conditions of Dera Ismail Khan. These results are in accordance with the findings of Jeuffroy *et al.* (2002) who revealed that growers are faced with the task of producing crops that achieve maximum yield, while limiting production costs, in order to maximize profit, minimize the environment impact, and produce a crop of extremely high quality that is demanded by today's consumers. Similar research work undertaken by Sturm *et al.* (1994) mentioned that the cost of treating urea with the AGROTAIN® would be in the range of US\$ 66.00 to 68.00 per (metric) t of urea. The present studies were also on the analogy of Shah *et al.* (2009) worked out that Benefit Cost Ratio (BCR) either on total cost container-1 bases (0.58: 1.00 and 0.76: 1.00) or

226 on solution chemicals cost bases (1.25: 1.00 and 2.76: 1.00) were found significantly greater
227 for Imai's recipe grown spinach crop.

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Table 1. Physico-Chemical Characteristics of Experimental Soil

Characteristics	Unit	Value
Bulk Density	g cm^{-3}	1.39
pH	-	8.10
ECe	dSm^{-1}	1.13
Lime (CaCO_3)	%	10.05
Organic Matter (OM)	%	0.92
Total Nitrogen	%	0.04
Available Phosphorus	$\mu\text{g g}^{-1}\text{ soil}$	7.10
Available Potassium	$\mu\text{g g}^{-1}\text{ soil}$	181.3
Clay	%	18.60
Silt	%	28.80
Sand	%	52.60
Textural Class	-	Sandy Clay Loam
Extractable Phosphorus	mg kg^{-1}	19.10
Exchangeable Potassium	mg kg^{-1}	189.20

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Table 2. Plant Height (cm), Number of Leaves, Fresh Weight (g), Dry Weight (g) and Biological Yield (g pot⁻¹) of Spinach

Treatments	Plant Height (cm)	Number of Leaves per Spinach	Fresh Weight (g)	Dry Weight (g)	Biological Yield (g pot ⁻¹)
T1	5.88 d	25.5 d	8.52 d	3.40 c	202 d
T2	7.17 cd	62.75 ab	15.79 bc	4.30 ab	216 bc
T3	8.33 cd	53.50 bc	14.12 c	4.28 ab	223 ab
T4	7.79 cd	54.50 abc	14.32 c	3.90 bc	213 c
T5	10.99 ab	54.00 abc	17.63 bc	4.43 ab	225 abc
T6	11.54 a	68.75 a	22.37 a	4.64 a	227 a
T7	8.67 bc	65.50 ab	19.41 ab	4.29 ab	215 bc
T8	9.66 abc	36.25 c	16.59 bc	4.17 ab	216 bc
T9	9.53 abc	58.25 abc	16.57 bc	4.63 a	219 abc
LSD0.05	2.5	15	4.16	0.7	10.6

Means followed by similar letter(s) do not differ significantly at P0.05

Table 3. Economize Nitrogen Fertilizer in Spinach Production

Treatments	Value of Produce (Rs. in Million)	Cost of Production (Rs. in Million)			Net Return (Rs. in Million)
		Cost of produce	Cost of Chemicals	Total	
T1	12.12	0.01	-	0.01	12.11
T2	12.96	0.01	0.01	0.02	12.94
T3	13.38	0.01	0.05	0.06	13.32
T4	12.78	0.01	0.26	0.27	12.51
T5	13.50	0.01	0.08	0.09	13.41
T6	13.62	0.01	0.19	0.20	13.42
T7	12.90	0.01	0.26	0.27	12.63
T8	12.96	0.01	0.47	0.48	12.48
T9	12.14	0.01	0.26	0.27	11.87

CONCLUSION

The study arrived at conclusion that almost all tested parameters were found instrumental especially dose of Urea@150 kg/ha + GA3 @60 gram/ha termed influential plant growth regulator for the sustained spinach productivity in hot climatic conditions of Dera Ismail Khan. There seems potential benefit of the combine application of Urea, Urease, Nitrification Inhibitors and Plant Growth Regulators on Spinach productivity.

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